Brit. J. vener. Dis. (1976) **52**, 136

Scanning electron microscopy of *Neisseria gonorrhoeae*

Age-induced changes in macro- and microstructure of virulent and avirulent colonies

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Summary

Virulent and avirulent gonococcal colonies were studied in the scanning electron microscope. Cells in virulent, in contrast to avirulent, colonies were found to be connected by numerous strands. After longer incubation periods type 2 colonies segregated out regions of cells virtually lacking these strands. Cells within these areas probably represent avirulent segregants. The microstructure of individual colonies revealed no topographical differences. In certain colonies large smooth spherical cells were seen, probably representing wall-deficient gonococcal variants.

Introduction

In routine diagnostic work with *Neisseria gonorrhoeae* two features of the bacteria are of crucial importance: their tendency to change colony morphology during incubation and their fragility during transport and cultivation. It was shown by Kellogg, Peacock, Deacon, Brown, and Pirkle (1963) that at least four different gonococcal colony types exist. A fifth type was described by Jephcott and Reyn (1971). Types 1 and 2 have been associated with gonococcal disease while the others have been isolated during subcultivation in the laboratory (Sparling and Yobs, 1967; Kellogg and others, 1963). The tendency to change colony morphology depends on strain characteristics and the interval of time between subcultivations. During prolonged growth periods on agar plates the tendency to segregate out non-virulent offspring is increased (Kellogg and others, 1963; Swanson, Sparks, Young, and King, 1975).

Gonococci are highly fragile organisms. One reason for this may be their high outer membrane permeability which may cause an increased sensitivity towards exogenous substances (Wolf-Watz, Elmros, Normark, and Bloom, 1975). Moreover, gonococci show a marked tendency to lyse (Morse and Bartenstein, 1974; Hebeler and Young, 1975). This lytic tendency may be associated with the formation of envelope defective cells ('L-forms'). Gonococcal 'L-forms' have been noted in both colonies (Dienes, Bandur, and Madoff, 1964) and during growth in liquid medium (Brookes and Hedén, 1967). Furthermore, 'L-forms' have been isolated from clinical specimens (Gnarpe, Wallin, and Forsgren, 1972). The significance of wall-deficient cells for gonococcal infection is unclear, but it may be suggested that diagnostic failures and cases failing to respond to penicillin are caused by such 'L-forms'.

Presented at the 28th General Assembly of the IUVDT, Malta, April, 1975
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Scanning electron microscopy

We have recently developed a method for the preparation of gonococcal colonies for scanning electron microscopy (SEM) (Elmros, Hörstedt, and Winblad, 1975).

The aim of the present report is to describe the use of this technique in the study of different colony types, colony changes induced by time, and individual cells within different parts of the colonies.

Findings and discussion

The five different colony types are depicted in Fig. 1 A to E. The virulent colony types 1 and 2 are smaller and higher, and show a more rounded edge than the avirulent types 3, 4, and 5. The explanation of this difference may be sought in the microstructure of each colony type. One characteristic feature of virulent colonies was an abundance of intercellular strands connecting neighbouring cells in the colony (Fig. 2). These strands were virtually lacking in the avirulent types. It is tempting to assume that the lack of these structures and consequent loss of cell-to-cell adhesions explains the radial growth tendency of avirulent colonies. The nature of these strands is at present not known but they may represent bundles of pili (Swanson, Kraus, and Gotschlich, 1971; Jephcott, Reyn, and Birch-Andersen, 1971).

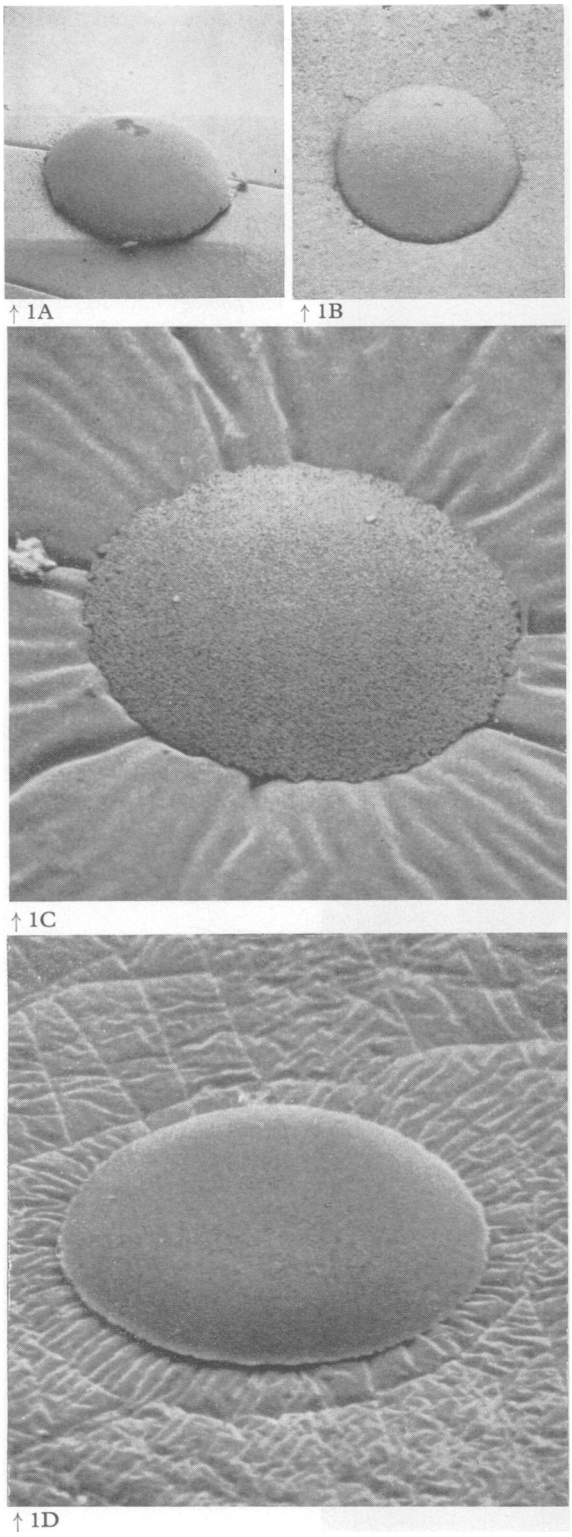


FIG. 1 *Low magnification scanning electron micrograph of the five gonococcal colony types incubated for 22 hrs on agar medium (GC Medium Base, Difco). All types were derivatives of strain No:* 82409/55, *obtained from Dr. Alice Reyn, Copenhagen. The preparation procedure for SEM was as described by Elmros, Hörstedt, and Winblad* (1975). 45° *angle* ×180. 1*A Colony type* 1 N. gonorrhoeae; 1 *B Type* 2; 1 *C Type* 3; 1 *D Type* 4; 1 *E Type* 5.

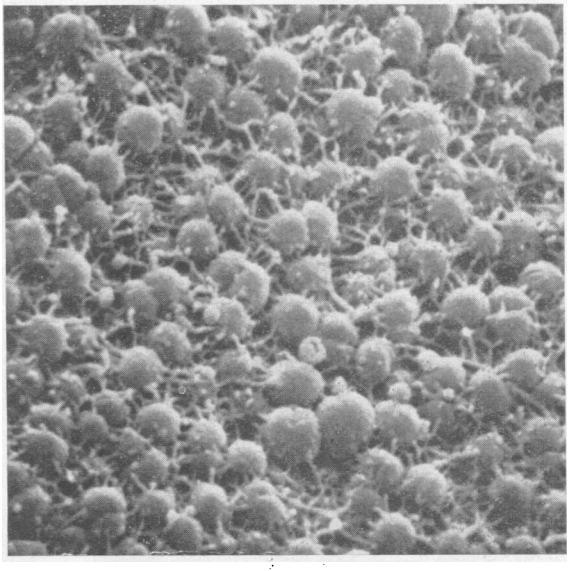


FIG. 2 *Type 2 colony incubated for 22 hrs. Note high frequency of intercellular strands. SEM* ×8,000.

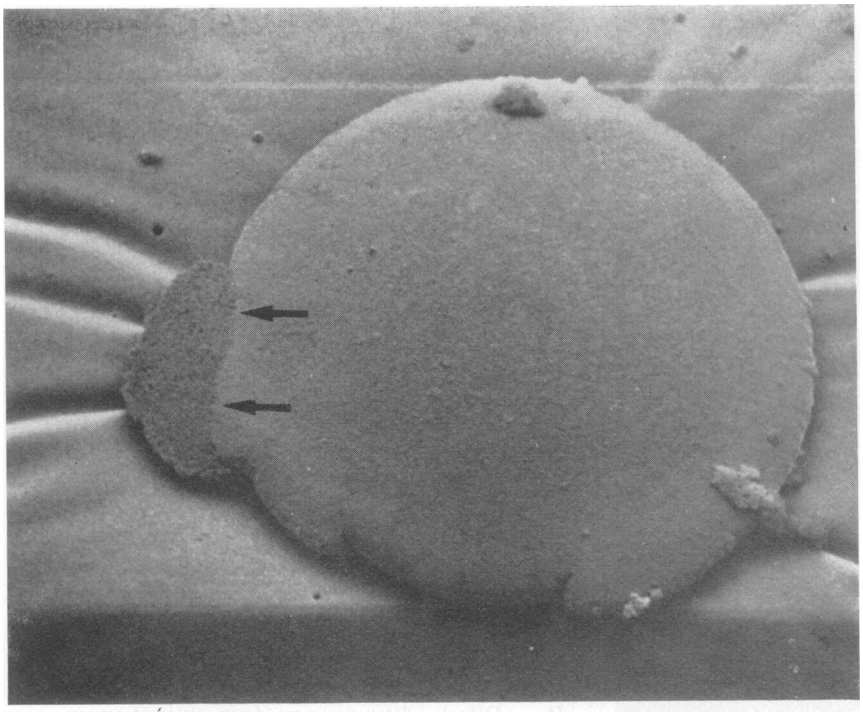
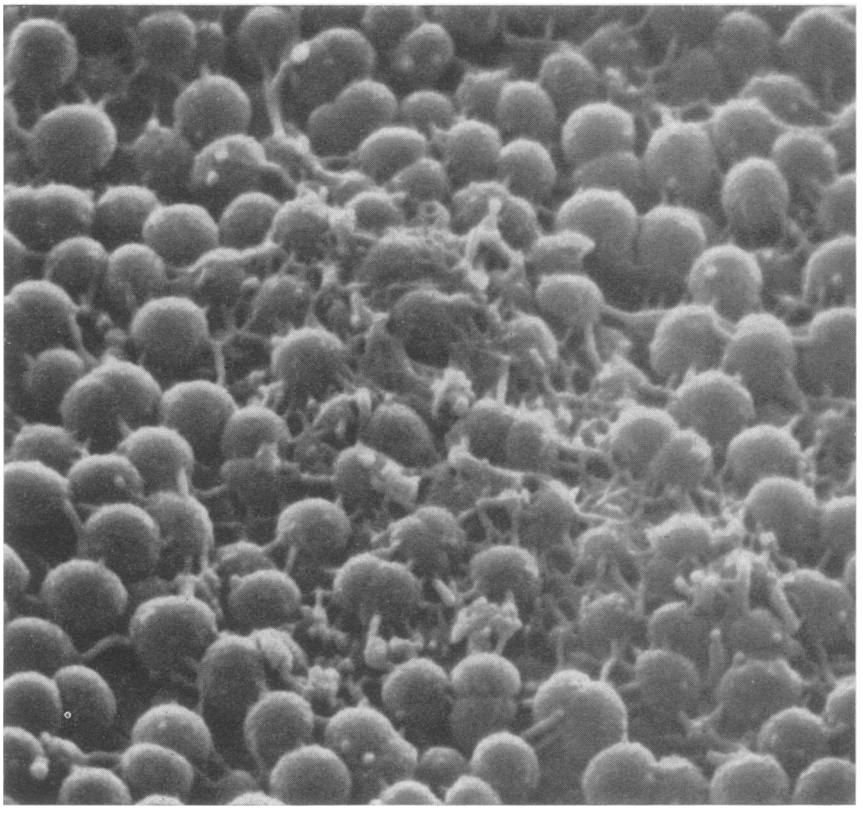


FIG. 3A *Type 2 colony incubated for 30 hrs. Regions with type 3 colony morphology have segregated out (arrows). Notice the smoother and more compact surface and the more vertical edge of the large type 2 colony. SEM* ×240.



B. *High magnification of an area at the top of the type 2 colony in 3A. Regionally, cells have segregated out virtually lacking intercellular strands. SEM* ×12,000.

Pili in enterobacteria are known to consist of proteins (Davis, Dulbecco, Eisen, Ginsberg, and Wood, 1973), and it was therefore somewhat surprising that proteases added to virulent colonies did not degrade these intercellular strands (Elmros and others, 1975).

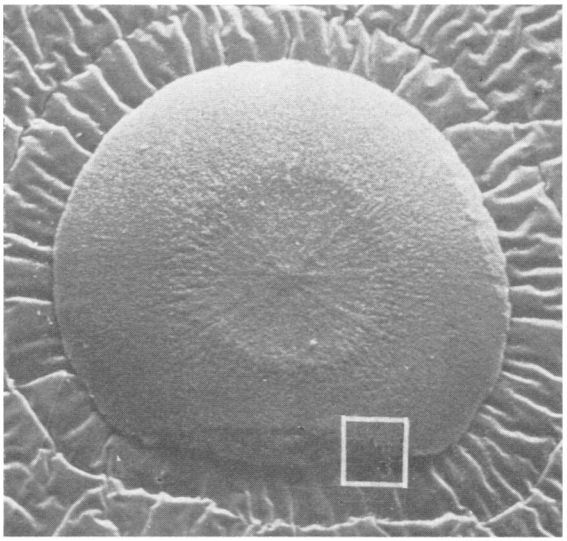
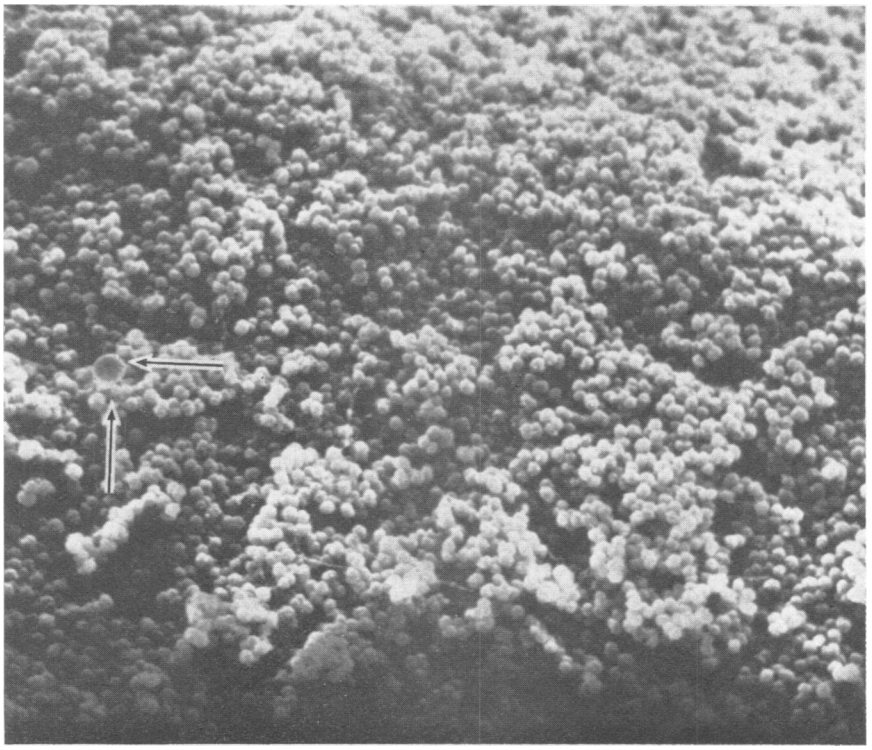


FIG. 4A *Low magnification of type 4 colony with fractured surface. SEM* ×120.

When virulent type 2 colonies were incubated for longer periods (about 30 hrs), new regions developed within or at the border of the colonies consisting of cells almost lacking the intercellular strands (Fig. 3 A and B). When such colonies were re-streaked avirulent type 3 colonies emerged. This marked tendency to segregate out avirulent offspring is at present not genetically understood. The reversion frequency appears higher than expected from a spontaneous mutation. It has been suggested but not proved that virulence in gonococci is mediated by extrachromosomal material (Mayer, Holmes, and Falkow, 1974; Stiffler, Lerner, Bohnoff, and Morello, 1975). If so it would explain the marked tendency to revert to avirulence. However, the conditions of cultivation may exert a selection pressure for avirulent colony types. The true reversion frequency is therefore hard to calculate. The fact that avirulent cells are segregated out not only at the periphery but also within the colony suggests that during equal conditions the growth of avirulent segregants *is* favoured.

It is well known that gonococci are very fragile, so that daily subcultivation is necessary. It was there-



B. *Medium magnification of the marked area in* 4A. *Note uniform appearance of cocci throughout the section. One large probably wall-deficient cell is seen (arrows). SEM* ×2,400.

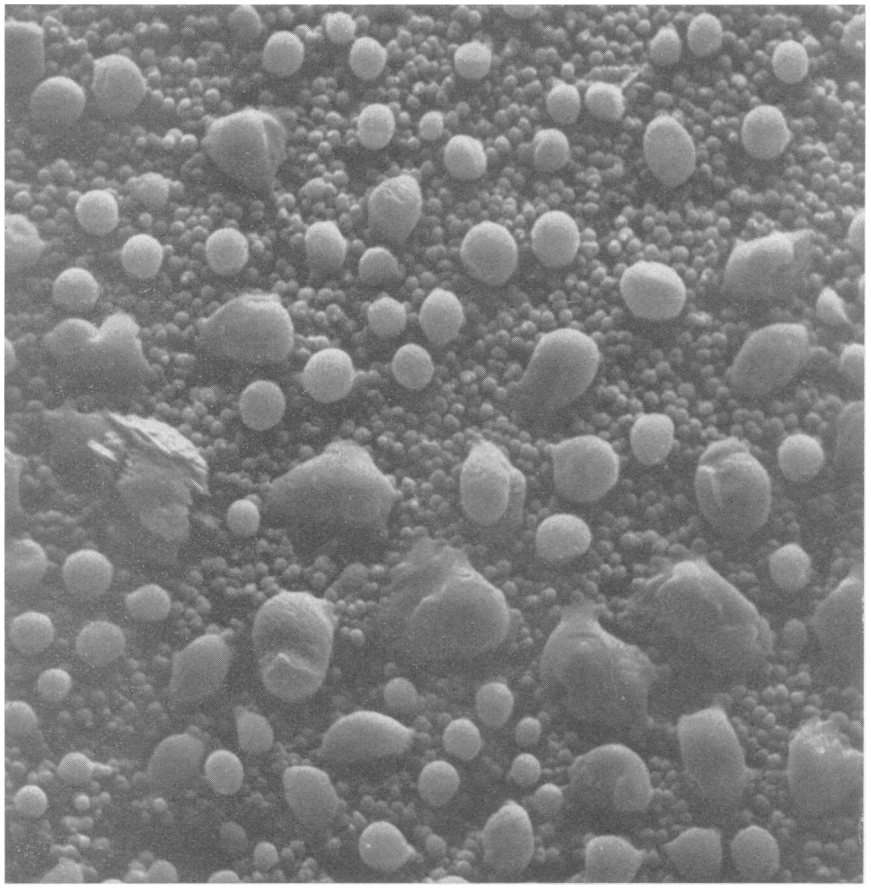


FIG. 5A. *Type 5 colony containing on the surface several large rounded, smooth obviously wall-deficient gonococci. SEM* ×2,400.

fore of interest to see if degenerated or wall-deficient cells occurred within a viable colony. A type 4 colony was broken after the critical-point drying procedure (Fig. 4 A and B). No difference in the cellular morphology or the intercellular relationship could be noted in the basal, middle, or top areas of the colony. Some large, probably wall-deficient, cells were seen within the colony.

No topographical variations in the morphology of the cells on the colony surface were observed. However, in certain colonies of both virulent and avirulent types, large bodies with a smooth surface were seen (Fig. 5 A and B). It is likely that these spherical cells represent wall-deficient gonococcal variants. The huge size is remarkable and must involve a considerable distention of the remaining envelope components. In contrast, Lawson and Douglas (1973) noted an irregular surface of the gonococcal L-forms. This could be due to a different preparation procedure. We have found that critical-point drying, the principle of which involves elimination of all surface tensions of ambient liquids, is the best method of preparing gonococcal colonies for SEM.

We wish to thank Gunnar Sandström and Per Hörstedt for skilful technical assistance. This work was supported by grants from the Swedish Medical Science Research Council (Dnr 4769) and Edvard Welander's Foundation.

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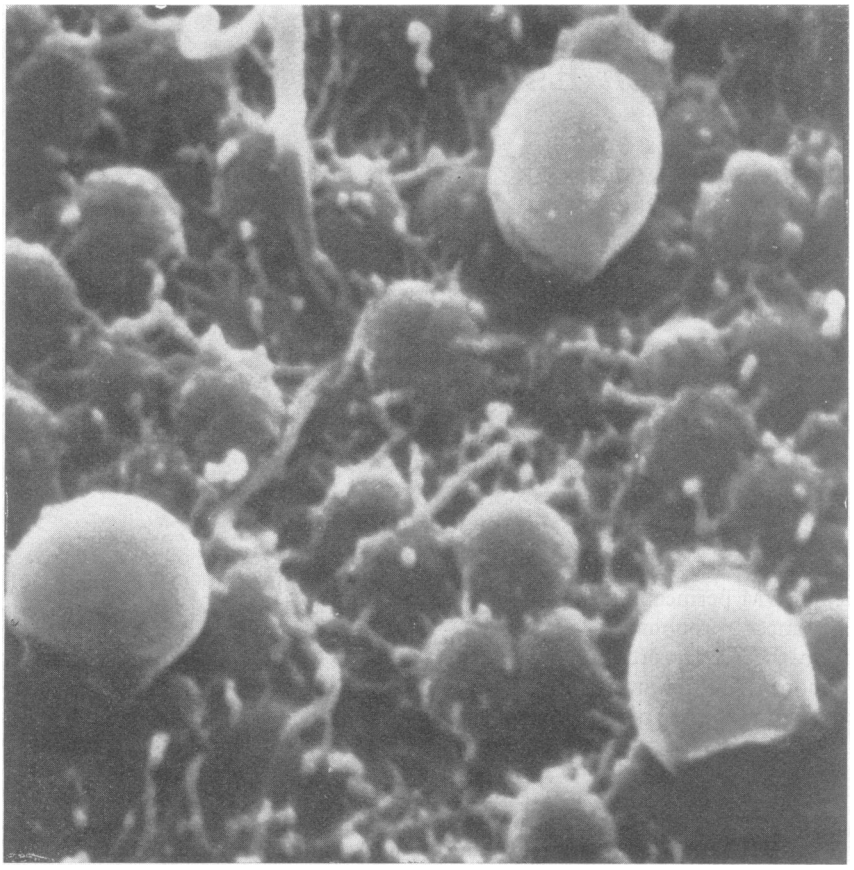

B. *High magnification of a type* 1 *colony. Three large probably wall-deficient cells are seen. SEM* × 24,000.

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